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NOVEL COMPOUNDS

Field of the Invention

The present invention discloses novel [1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one derivatives together with processes for their preparation, pharmaceutical compositions comprising them and their use in therapy.

Background of the Invention

Chemokines play an important role in immune and inflammatory responses in various diseases and disorders, including asthma and allergic diseases and inflammatory bowel disease (IBD), as well as autoimmune pathologies such as rheumatoid arthritis and atherosclerosis. These small secreted molecules are a growing superfamily of 8-14 kDa proteins characterised by a conserved four cysteine motif. The chemokine superfamily can be divided into two main groups exhibiting characteristic structural motifs, the Cys-X-Cys (C-X-C) and Cys-Cys (C-C) families. These two groups are distinguished on the basis of a single amino acid insertion between the NH-proximal pair of cysteine residues and sequence similarity.

The C-X-C chemokines include several potent chemoattractants and activators of neutrophils such as interleukin-8 (CXCL8) and neutrophil-activating peptide 2 (CXCL7).

The C-C chemokines include potent chemoattractants of monocytes and lymphocytes but not neutrophils. Examples include human monocyte chemotactic proteins 1-3 (CCL2, CCL7 and CCL8), RANTES (CCL5), eotaxin (CCL11) and the macrophage inflammatory proteins 1α and 1β (CCL3 and CCL4).

There is also a third chemokine family based upon the structural motif Cys-X_3-Cys

($C\text{-}X_3\text{-}C$). This $C\text{-}X_3\text{-}C$ family is distinguished from the C-X-C and C-C families on the basis of having a triple amino acid insertion between the NH-proximal pair of cysteine residues. CX_3CL1 (also known as fractalkine) is a potent chemoattractant and activator of microglia in the central nervous system as well as of monocytes, T cells, NK cells and mast cells.

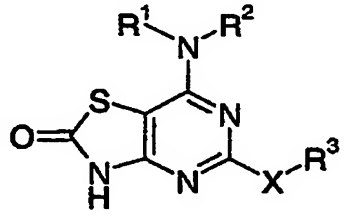
Studies have demonstrated that the actions of the chemokines are mediated by subfamilies of G protein-coupled receptors. In particular, the actions of CX_3CL1 are mediated by the CX_3CR1 receptor.

WO 01/25242 discloses certain [1,3]thiazolo[4,5-d]pyrimidin-2(3H)-one derivatives that are useful as antagonists of receptors linked to the C-X-C and C-C chemokine families, particularly as antagonists of the CXCR2 receptor.

The present invention relates to a group of compounds that are structurally similar to, but nevertheless generically distinct from, the compounds disclosed in WO 01/25242. The compounds of the present invention display surprisingly useful properties as antagonists of the CX_3CR1 receptor.

Disclosure of the invention

The present invention provides compounds of formula (I)



(I)

wherein:

R^1 and R^2 independently represent H, C1 to 8 alkyl, C2 to 8 alkenyl, C2 to 8 alkynyl or C3 to 7 saturated or partially unsaturated cycloalkyl; the latter four groups being optionally further substituted by one or more groups selected independently from OH, C1 to 6 alkoxy, CH_2OR^4, NR^5R^6, CO_2R^7 and $CONR^8R^9$;

R^3 represents C1 to 6 alkyl, C2 to 6 alkenyl, C2 to 6 alkynyl or C3 to 7 saturated or partially unsaturated cycloalkyl; said alkyl, alkenyl or alkynyl chain optionally including a O, NR^{10} or S atom in the chain; said alkyl, alkenyl, alkynyl or cycloalkyl group being optionally substituted by phenyl or a 5 or 6 membered heteroaromatic ring containing 1 to 3 heteroatoms selected independently from O, S and N; said phenyl or heteroaromatic ring being optionally further substituted by one or more groups selected independently from halogen, C1 to 4 alkyl, OH, C1 to 4 alkoxy, CN, $CO2R^{11}$, $NR^{12}R^{13}$, $CONR^{14}R^{15}$, SO_2R^{16}, $NR^{17}SO_2R^{18}$ and $SO_2NR^{19}R^{20}$;

X represents O or S(O);

R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{20} independently represent H or C1 to 6 alkyl;

and pharmaceutically acceptable salts thereof.

The compounds of formula (I) may exist in enantiomeric and/or tautomeric forms. It is to be understood that all enantiomers, diastereomers, racemates, tautomers and mixtures thereof are included within the scope of the invention.

Unless otherwise indicated, the term "C1 to 8 alkyl" referred to herein denotes a straight or branched chain alkyl group having from 1 to 8 carbon atoms. Examples of such groups

include methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, pentyl and hexyl. The terms "C1 to 6 alkyl" and "C1 to 4 alkyl" are to be interpreted analogously.

Unless otherwise indicated, the term "C2 to 8 alkenyl" referred to herein denotes a straight or branched chain alkyl group having from 2 to 8 carbon atoms and containing one carbon-carbon double bond. The term "C2 to 6 alkenyl" is to be interpreted analogously.
Unless otherwise indicated, the term "C2 to 8 alkynyl" referred to herein denotes a straight or branched chain alkyl group having from 2 to 8 carbon atoms and containing one carbon-carbon triple bond. The term "C2 to 6 alkenyl" is to be interpreted analogously.

Unless otherwise indicated, the term "C3 to 7 saturated or partially unsaturated cycloalkyl" referred to herein denotes a 3 to 7 membered non-aromatic carbocyclic ring optionally incorporating one or more double bonds. Examples include cyclopropyl, cyclopentyl, cyclopentenyl, cyclohexyl and cyclohexenyl.

Unless otherwise indicated, the term "C1 to 6 alkoxy " referred to herein denotes an oxygen substituent bonded to a straight or branched chain alkyl group having from 1 to 6 carbon atoms. Examples of such groups include methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, i-butoxy and s-butoxy. The term "C1 to 4 alkoxy" is to be interpreted analogously.

Unless otherwise indicated, the term "halogen" referred to herein denotes fluorine, chlorine, bromine and iodine.

Examples of a five or six membered heteroaromatic ring containing 1 to 3 heteroatoms independently selected from O, S and N include furan, thiophene, pyrrole, oxazole, oxadiazole, isoxazole, imidazole, thiazole, triazole, thiadiazole, pyridine, pyrimidine and pyrazine.

In the definition of R^3, the expression "said alkyl, alkenyl or alkynyl chain optionally including a O, NR^{10} or S atom in the chain" embraces a straight or branched chain

arrangement of 1 to 6 carbon atoms in which, where chemically feasible, the carbon chain is interrupted by, or terminates in, an O, S or NR^{10} atom. The definition thus includes, for example, methylene, ethylene, propylene, hexamethylene, ethylethylene, $-CH_2CH_2O-CH_2-$, $-CH_2CH_2O-CH_2-CH_2-$, $-CH_2CH_2S-$ and $-CH_2CH_2NR^{10}-$.

In one embodiment, X represents O. In another embodiment, X represents S(O).

In one embodiment, R^1 and R^2 independently represent H, optionally substituted C1 to 8 alkyl or optionally substituted C3 to 7 cycloalkyl.

In another embodiment, R^1 represents H or CH_3. In another embodiment, R^1 represents H.

In another embodiment R^2 represents optionally substituted C1 to 8 alkyl or optionally substituted C3 to 7 cycloalkyl. In another embodiment, R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4.

In one embodiment, R^3 represents optionally substituted C1 to 6 alkyl that optionally includes an O atom in the chain. In another embodiment, R^3 represents C1 to 6 alkyl optionally including an O atom in the chain and substituted by optionally substituted phenyl. In another embodiment, R^3 represents C1 to 2 alkyl substituted by phenyl; said phenyl being optionally substituted by halogen, C1 to 6 alkoxy or CN.

In one embodiment, X represents O, R^1 represents H or CH_3; R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4; and R^3 represents C1 to 6 alkyl substituted by optionally substituted phenyl.

In another embodiment, X represents O, R^1 represents H; R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4; and R^3 represents

C1 to 2 alkyl substituted by phenyl; said phenyl being optionally substituted by halogen, C1 to 6 alkoxy or CN.

In one embodiment, X represents S(O), R^1 represents H or CH_3; R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4; and R^3 represents C1 to 6 alkyl substituted by optionally substituted phenyl.

In another embodiment, X represents S(O), R^1 represents H; R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4; and R^3 represents C1 to 2 alkyl substituted by phenyl; said phenyl being optionally substituted by halogen, C1 to 6 alkoxy or CN.

Particular compounds of formula (I) include:

5-(benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}-5-[(3-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}-5-(2-phenylethoxy)[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-(benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}-5-{[(1*S*)-1-phenylethyl]oxy}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

N-(3-{[(7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)-*N*-methylmethanesulfonamide;

N-(3-{[(7-{[(1*R*)-1-(hydroxymethyl)-2-methylpropyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)methanesulfonamide;

5-(benzyloxy)-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[1-(hydroxymethyl)cyclopentyl]amino}-5-[(2-methylbenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[1-(hydroxymethyl)cyclopentyl]amino}-5-[(3-methylbenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[(2-chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[(3-chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[(4-chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[1-(hydroxymethyl)cyclopentyl]amino}-5-[(2-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[1-(hydroxymethyl)cyclopentyl]amino}-5-[(3-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

4-{[(7-{[1-(hydroxymethyl)cyclopentyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}benzonitrile;

(*R*,*S*)-7-[[1-(hydroxymethyl)cyclopentyl]amino]-5-(1-phenylethoxy)-thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

7-{[1-(hydroxymethyl)cyclopentyl]amino}-5-{[(1*S*)-1-phenylethyl]oxy}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-{[2-(3-chlorophenyl)ethyl]-(R_S,S_S)-sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-{[2-(2-bromophenyl)ethyl]-(R_S,S_S)-sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[(2,3-difluorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[benzyl-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[(2-chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

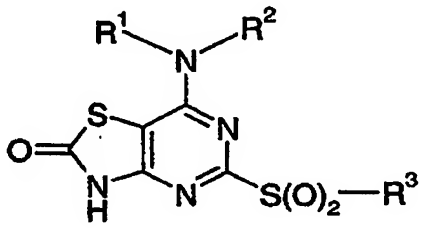
5-[(4-chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

5-[benzyl-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-2-methylpropyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one;

and pharmaceutically acceptable salts thereof.

According to the invention, we further provide a process for the preparation of a compound of formula (I), or a pharmaceutically acceptable salt, enantiomer or racemate thereof which comprises:

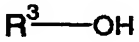
(a) when X in formula (I) represents O, reaction of a compound of formula (II)



(II)

wherein R^1, R^2 and R^3 are as defined in formula (I);

with a compound of formula (III)

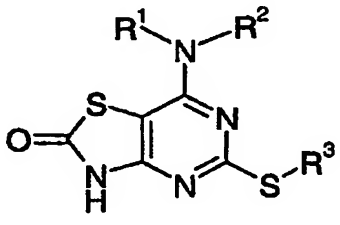


(III)

wherein R^3 is as defined in formula (I) and is independent of the R^3 group in formula (II);

or

(b) when X in formula (I) represents S(O), oxidation of a compound of formula (IV)



(IV)

wherein R^1, R^2 and R^3 are as defined in formula (I); with one equivalent of an oxidising agent;

and where necessary converting the resultant compound of formula (I), or another salt thereof, into a pharmaceutically acceptable salt thereof; or converting the resultant compound of formula (I) into a further compound of formula (I); and where desired converting the resultant compound of formula (I) into an optical isomer thereof.

In process (a), the reactants (II) and (III) are coupled together in a suitable inert organic solvent such as tetrahydrofuran, benzene, toluene or N-methylpyrrolidine. The reaction is performed in the presence of an added base such as sodium hydride, butyl lithium or lithium diisopropylamide. The reaction is conducted at a suitable temperature, normally between room temperature and the boiling point of the solvent. The reaction is generally continued for a period of about one hour to one week, or until analysis indicates that formation of the required product is complete.

In process (b), the compound is oxidised using one equivalent of a suitable oxidising agent such as those known in the art for the oxidation of sulphides into sulphoxides. A preferred oxidant is oxone. The reaction is generally conducted at ambient temperature and in a suitable solvent such as methanol or aqueous acetonitrile.

Compounds of formula (I) and intermediate compounds thereto may be prepared as such or in protected form. Protecting groups that are suitable for particular functional groups and details of processes for adding and removing such protecting groups are, in general, well known in the art. See, for example, "Protective Groups in Organic Synthesis", 3rd Edition (1999) by Greene and Wuts.

The present invention includes compounds of formula (I) in the form of salts. Suitable salts include those formed with organic or inorganic acids or organic or inorganic bases. Such salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable acids or bases may be of utility in the preparation and purification of the compound in question. Thus, preferred acid addition salts include those formed from hydrochloric, hydrobromic, sulphuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic,

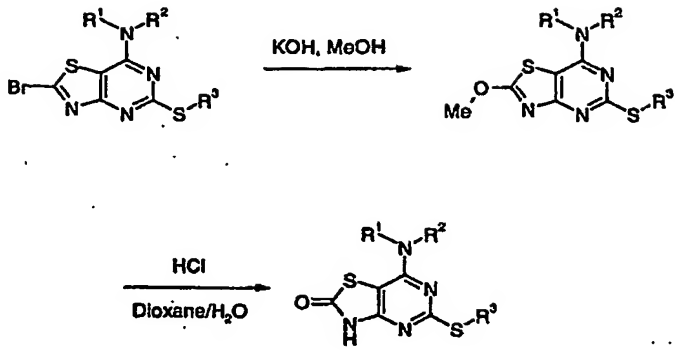
succinic, fumaric, maleic, methanesulphonic and benzenesulphonic acids. Preferred base addition salts include those in which the cation is sodium, potassium, calcium, aluminium, lithium, magnesium, zinc, choline, ethanolamine or diethylamine.

Salts of compounds of formula (I) may be formed by reacting the free base, or a salt, enantiomer or racemate thereof, with one or more equivalents of the appropriate acid. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, for example, water, dioxan, ethanol, tetrahydrofuran or diethyl ether, or a mixture of solvents, which may be removed *in vacuo* or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange resin.

Sulphone derivatives of formula (II) may be prepared by oxidation of the corresponding sulphides of formula (IV) using two or more equivalents of an oxidising agent such as oxone.

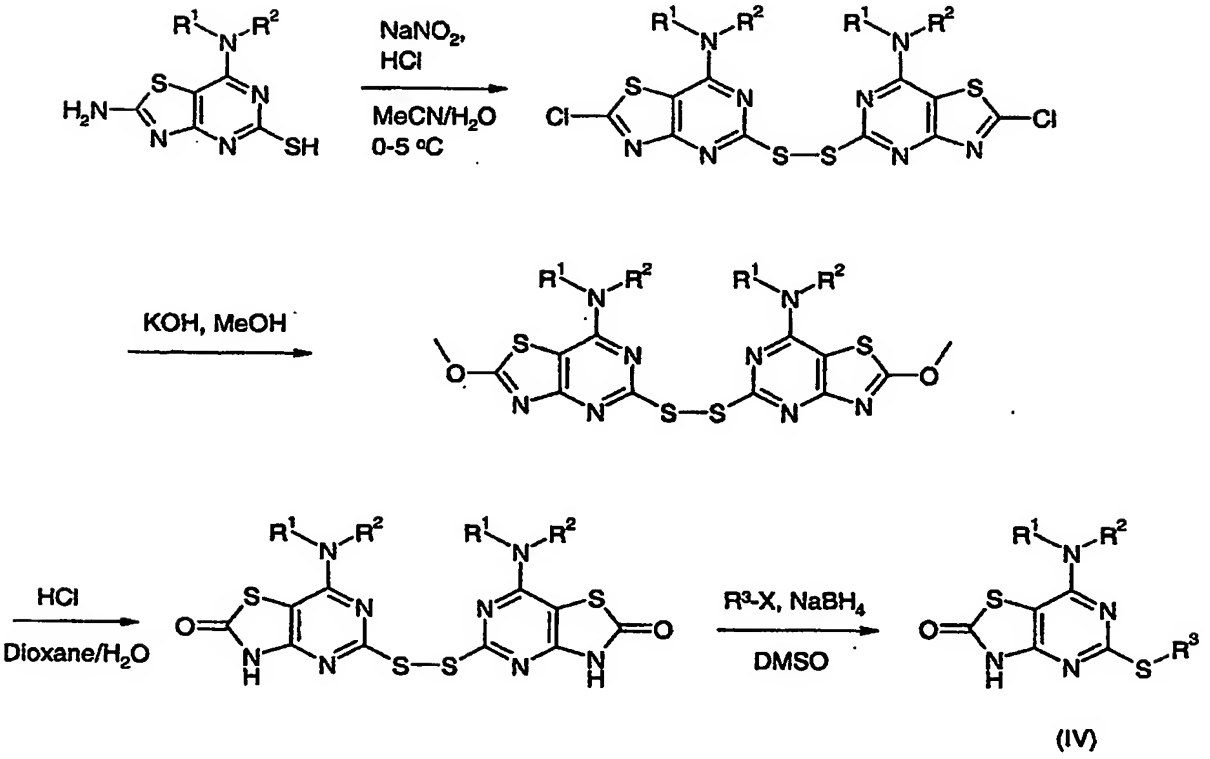
In general, compounds of formula (IV) may be prepared using known methods that will be readily apparent to the man skilled in the art. Some such methods are illustrated in Schemes 1 to 3:

Scheme 1



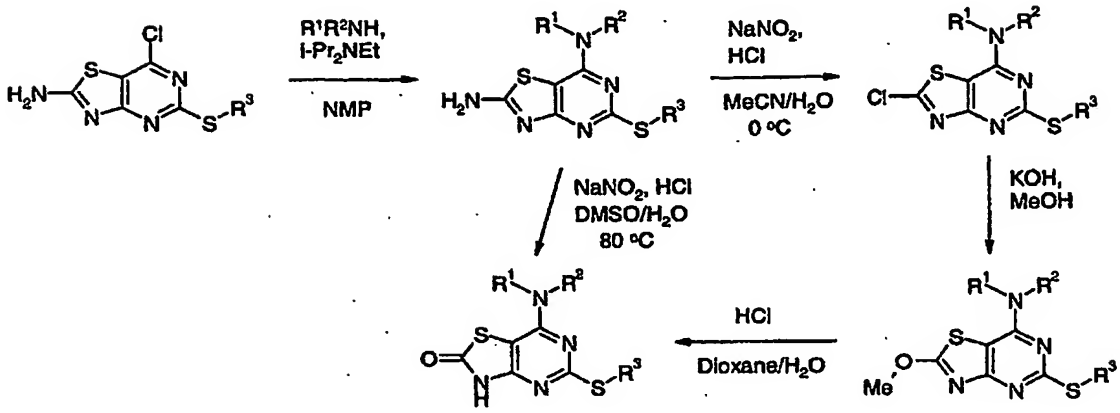
(IV)

Scheme 2



(IV)

Scheme 3



(IV)

Intermediate compounds may be used as such or in protected form. Protecting groups and details of processes for their removal may be found by reference to the standard text "Protective Groups in Organic Synthesis", 3rd Edition (1999) by Greene and Wuts.

The compounds of the invention and intermediates thereto may be isolated from their reaction mixtures and, if necessary further purified, by using standard techniques.

The compounds of formula (I) may exist in enantiomeric forms. Therefore, all enantiomers, diastereomers, racemates and mixtures thereof are included within the scope of the invention. The various optical isomers may be isolated by separation of a racemic mixture of the compounds using conventional techniques, for example, fractional crystallisation, or HPLC. Alternatively, the various optical isomers may be prepared directly using optically active starting materials.

Intermediate compounds may also exist in enantiomeric forms and may be used as purified enantiomers, diastereomers, racemates or mixtures.

The compounds of formula (I), and their pharmaceutically acceptable salts are useful because they possess pharmacological activity as antagonists of the CX_3CR1 receptor. In particular, when compared to similar sulphide derivatives disclosed in WO 01/25242, the ether [formula (I); X = O] and sulphoxide [formula (I); X = S(O)] derivatives of the present invention possess significantly improved solubility profiles.

In one aspect the present invention provides a compound of formula (I) or a pharmaceutically acceptable salt thereof, for use as a medicament.

In another aspect the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament, for the treatment or prophylaxis of diseases or conditions in which antagonism of the CX_3CR1 receptor is beneficial.

In another aspect the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament, for the treatment or prophylaxis of neurodegenerative disorders, demyelinating disease, atherosclerosis or pain.

According to the invention, there is also provided a method of treating, or reducing the risk of, diseases or conditions in which antagonism of the CX_3CR1 receptor is beneficial which comprises administering to a person suffering from or at risk of, said disease or condition, a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

There is also provided a method of treating, or reducing the risk of, neurodegenerative disorders, demyelinating disease, atherosclerosis or pain in a person suffering from or at risk of, said disease or condition, wherein the method comprises administering to the

person a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

In another aspect the invention provides a pharmaceutical formulation comprising a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, for use in the treatment or prophylaxis of diseases or conditions in which antagonism of the CX_3CR1 receptor is beneficial.

In another aspect the invention provides a pharmaceutical formulation comprising a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, for use in the treatment or prophylaxis of neurodegenerative disorders, demyelinating disease, atherosclerosis or pain.

The compounds of formula (I) and their pharmaceutically acceptable salts are indicated for use in the treatment or prophylaxis of diseases or conditions in which modulation of activity at the CX_3CR1 receptor is desirable. In particular, the compounds are indicated for use in the treatment of neurodegenerative disorders or demyelinating disease in mammals including man. The compounds are also indicated to be useful in the treatment of pain, rheumatoid arthritis, osteoarthritis, stroke, atherosclerosis and pulmonary arterial hypertension.

Conditions that may be specifically mentioned are: neurodegenerative diseases and dementia disorders, for example, Alzheimer's disease, amyotrophic lateral sclerosis and other motor neuron diseases, Creutzfeldt-Jacob's disease and other prion diseases, HIV encephalopathy, Huntington's disease, frontotemporal dementia, Lewy body dementia and vascular dementia; polyneuropathies, for example, Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, multifocal motor neuropathy and plexopathies; CNS demyelination, for example, acute disseminated/haemorrhagic encephalomyelitis and subacute sclerosing panencephalitis; neuromuscular disorders, for example, myasthenia gravis

and Lambert-Eaton syndrome; spinal disorders, for example, tropical spastic paraparesis and stiff-man syndrome; paraneoplastic syndromes, for example, cerebellar degeneration and encephalomyelitis; CNS trauma; and migraine.

Prophylaxis is expected to be particularly relevant to the treatment of persons who have suffered a previous episode of, or are otherwise considered to be at increased risk of, the disease or condition in question. Persons at risk of developing a particular disease or condition generally include those having a family history of the disease or condition, or those who have been identified by genetic testing or screening to be particularly susceptible to developing the disease or condition.

The compounds of the invention are also indicated for use in the treatment of inflammatory bowel disease (IBD), for example, Crohn's disease and ulcerative colitis, by inducing remission and/or maimtaining remission of IBD.

For the above mentioned therapeutic indications, the dosage administered will, of course, vary with the compound employed, the mode of administration and the treatment desired. However, in general, satisfactory results are obtained when the compounds are administered at a dosage of the solid form of between 1 mg and 2000 mg per day.

The compounds of formula (I) and pharmaceutically acceptable derivatives thereof, may be used on their own, or in the form of appropriate pharmaceutical compositions in which the compound or derivative is in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier. Administration may be by, but is not limited to, enteral (including oral, sublingual or rectal), intranasal, intravenous, topical or other parenteral routes. Conventional procedures for the selection and preparation of suitable pharmaceutical formulations are described in, for example, "Pharmaceuticals - The Science of Dosage Form Designs", M. E. Aulton, Churchill Livingstone, 1988. The pharmaceutical composition preferably comprises less than 80% and more preferably less than 50% of a compound of formula (I), or a pharmaceutically acceptable salt thereof.

There is also provided a process for the preparation of such a pharmaceutical composition that comprises mixing the ingredients.

The invention is illustrated, but in no way limited, by the following examples:

General Procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 7 Tesla 300 MHz instrument, or a Bruker Avance 400 MHz instrument using the solvent indicated. Chemical shifts are given in ppm down- and upfield from tetramethylsilane (TMS). Resonance multiplicities are denoted s, d, t, m, br and app for singlet, doublet, triplet, multiplet, broad and apparent, respectively. Mass spectra (MS) were recorded on a Finnigan SSQ7000 TSP or a Finnigan SSQ710 DI/EI instrument, or on a single quadropole mass spectrometer, ZMD (Waters), using an electrospray ion source operated in a positive mode. The ion spray voltage was +3 kV and the mass spectrometer was scanned from *m/z* 100 – 900 with a scan time of 0.85s. LC-MS was performed with a Waters 2790 LC-system equipped with a Waters Xterra™ MS C_8 (2.5 µm x 30 mm) column, a Waters 996 photodiode array detector and a Micromass ZMD. High pressure liquid chromatography (HPLC) assays were performed using a Hewlett Packard 1100 Series HPLC system equipped with a Zorbax SB-C_8 (4.6 mm x 15 cm) column. Preparative high pressure liquid chromatography (prep HPLC) separations were performed on an automated Gilson (model 170) using an Xterra C_{18} (19 mm x 30 cm) column, and using a gradient of A (water 95%, containing NH_4OAc (0.01 M), and 5% CH_3CN) and B (CH_3CN) as eluent. Column chromatography was performed using silica gel 60 (230-400 mesh ASTM, Merck) and thin layer chromatography (TLC) was performed on TLC precoated plates, silica gel 60 F_{254} (Merck).

Example 1 5-(Benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) (2*R*)-2-({2-Chloro-5-[(2,3-difluorobenzyl)thio][1,3]thiazolo[4,5-*d*]pyrimidin-7-yl}amino)-4-methylpentan-1-ol

(2*R*)-2-({2-Amino-5-[(2,3-difluorobenzyl)thio][1,3]thiazolo[4,5-*d*]pyrimidin-7-yl}amino)-4-methylpentan-1-ol (WO 00/09511) (20.0 g, 47 mmol) was dissolved in conc. HCl (750 mL). CH_3CN (600 mL) and water (350 mL) were added and the mixture was cooled to 0 °C. A solution of $NaNO_2$ (3.24 g, 94 mmol) in water (20 mL) was then added portionwise, and the mixture was stirred at 0 °C for 1.5 h. The yellow solid which had formed was collected by filtration, washed with water and dried to give 16.3 g (88%) of the title compound as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ 8.15 (d, 1H), 7.42-7.28 (m, 2H), 7.20-7.10 (m, 1H), 4.50 (b s, 1H), 4.46 (app t, 2H), 4.38-4.25 (m, 1H), 3.42 (app q, 2H), 1.67-1.54 (m, 1H), 1.53-1.42 (m, 1H), 1.41-1.32 (m, H), 0.88 (d, 3H), 0.83 (d, 3H);
MS (ESI$^+$) *m/z* 445 $[M+H]^+$.

(b) (2*R*)-2-({5-[(2,3-Difluorobenzyl)thio]-2-methoxy[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl}amino)-4-methylpentan-1-ol

The product from step (a) (10.75 g, 24.4 mmol) was dissolved in MeOH and solid potassium hydroxide (2.74 g, 48.8 mmol) was added. The mixture was heated to 55 °C for 1 h, cooled to RT and then neutralized with 2N HCl. MeOH was removed by evaporation *in vacuo*, water was added to the residue and the crude product was collected by filtration. Recrystallization from CH_3CN gave title compound (9.25 g; 88%) as a pale orange solid.
^{1}H NMR (DMSO-d_6) δ 7.60 (d, 1H), 7.40-7.28 (m, 2H), 7.20-7.10 (m, 1H), 4.74 (t, 1H), 4.44 (app q, 2H), 4.29 (b s, 1H), 4.16 (s, 3H), 3.9-3.35 (m, 2H, partially under the water peak), 1.65-1.52 (m, 1H), 1.50-1.32 (m, 2H), 0.87 (d, 3H), 0.82 (d, 3H);
MS (ESI$^+$) *m/z* 441 $[M+H]^+$.

(c) 5-[(2,3-Difluorobenzyl)thio]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from step (b) (8.83 g, 20.0 mmol) was suspended in dioxane (300 mL). Conc. HCl (1.5 mL) and water (1 mL) were added and the mixture was heated to 50 °C for 15 h. Solvents were removed in vacuo and the residue was suspended in CH_3CN (300 mL). The

off white solid was filtered off, washed with CH_3CN and dried to afford 7.92 g (90%) of the title compound.

^1H NMR (DMSO-d_6) δ 12.43 (br s, 1H), 7.45-7.27 (m, 3H), 7.20-7.08 (m, 1H), 4.46 (b s, 2H), 4.39 (1H, under the water peak), 4.26 (br s, 1H), 3.42-3.28 (m, 2H), 1.62-1.50 (m, 1H), 1.48-1.39 (m, H), 1.38-1.28 (m, 1H), 0.86 (d, 3H), 0.81 (d, 3H);

MS (ESI$^+$) *m/z* 427 $[M+H]^+$.

(d) 5-[(2,3-Difluorobenzyl)sulfonyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from step (c) (2.0 g, 4.68 mmol) was dissolved in CH_3CN (240 mL) and water (160 mL). Potassium peroxymonosulfate (Oxone, 6.32 g, 10.30 mmol) was added and the resulting inhomogeneous mixture was stirred at RT for 24 h. Sodium thiosulphate solution was added and the CH_3CN was evaporated *in vacuo*. The residue was poured onto ice and the precipitate was collected by filtration, washed with water and dried *in vacuo* at 40 °C overnight resulting in 1.76 g (82%) of the title compound as an off-white solid.

^1H NMR (DMSO-d_6) δ 13.10 (br s, 1H), 7.52-7.40 (m, 2H) 7.28-7.18 (m, 2H), 5.0-4.85 (app t, 2H), 4.77 (br s, 1H), 4.29 (br s, 1H), 3.34 (br s, 2H), 1.65-1.51 (m, 1H), 1.50-1.31 (m, 2H), 0.88 (d, 3H), 0.85 (d, 3H);

MS (ESI$^+$) *m/z* 459 $[M+H]^+$.

(e) 5-(Benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Solid sodium hydride (17 mg, 0.7 mmol) was added to a stirred solution of benzyl alcohol (76 mg, 0.7 mmol) in dry benzene (5 mL) at 0 °C. The solution was allowed to reach RT over 15 min. The product from step (d) (46 mg, 0.1 mmol) was added as a solid, and the mixture was heated to reflux for 1 h. After cooling to RT, the reaction was quenched by the addition of of saturated aqueous NH_4Cl (1 mL). The mixture was partitioned between THF (10 mL) and water (10 mL). The organic phase was separated, dried over Na_2SO_4 and evaporated *in vacuo*. The oily residue was purified by preparative HPLC, to give the title compound as a crystalline solid (6.0 mg, 16% yield).

^{1}H NMR (DMSO-d_6) δ 12.05 (br s, 1H), 7.52-7.24 (m, 5H), 5.95 (d, 1H), 5.02 (s, 2H), 4.77-4.29 (m, 2H), 3.38-3.30 (m, 2H), 1.63 (m, 1H), 1.50-1.32 (m, 2H), 0.89 (d, 3H), 0.83 (d, 3H);
MS (ESI$^+$) *m/z* 375 $[M+H]^+$.

The compounds of Examples 2 and 3 were prepared using the general method of Example 1, step (e), but replacing benzyl alcohol with the appropriate alcohol.

Example 2 7-{[(1*R*)-1-(Hydroxymethyl)-3-methylbutyl]amino}-5-[(3-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (4.8 mg, 12% yield).
^{1}H NMR (DMSO-d_6) δ 12.75 (br s, 1H), 7.44-7.24 (m, 2H), 6.91 (s, 1H), 6.84 (d, 1H), 5.90 (d, 1H), 5.22 (s, 2H), 4.70-4.31 (m, 2H), 3.48 (s, 3H), 3.40-3.30 (m, 2H), 1.62 (m, 1H), 1.50-1.31 (2H), 0.88 (d, 3H), 0.83 (d, 3H);
MS (ESI$^+$) *m/z* 405 $[M+H]^+$.

Example 3 7-{[(1*R*)-1-(Hydroxymethyl)-3-methylbutyl]amino}-5-(2-phenylethoxy)[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (8.1 mg, 21% yield).
^{1}H NMR (DMSO-d_6) δ 12.87 (br s, 1H), 7.44-7.16 (m, 5H), 5.90 (d, 1H), 4.81 (t, 2H), 4.58-4.30 (m, 2H), 3.42-3.32 (m, 2H), 3.29 (t, 2H), 1.63 (m, 1H), 1.52-1.31 (m, 2H), 0.88 (d, 3H), 0.80 (d, 3H);
MS (ESI$^+$) *m/z* 389 $[M+H]^+$.

Example 4 5-(Benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) (2*R*)-2-{[2-Amino-5-(benzylthio)[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}pentan-1-ol
5-(Benzylthio)-7-chloro[1,3]thiazolo[4,5-*d*]pyrimidin-2-amine (WO 00/09511) (2.03 g, 6.57 mmol) was dissolved in 1-methyl-2-pyrrolidinone (NMP) (12 mL). *N*-Ethyl-*N*,*N*-diisopropylamine (DIPEA) (2.25 mL, 13.1 mmol) and 2-amino-(2*R*)-1-pentanol (1.19 g,

11.5 mmol) were added and the mixture was heated to 110 °C for 4 days. After cooling to RT, the mixture was poured into water (200 mL). The yellow solid was collected by filtration, washed with water and used for the next step without further purification (yield 80%).

MS (ESI$^+$) *m/z* 376 $[M+H]^+$.

(b) (2*R*)-2-{[5-(Benzylthio)-2-chloro[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}pentan-1-ol

The product from step (a) (2.46 g, 6.57 mmol) was dissolved in CH_3CN (70 mL). Sodium nitrite (1.36 g, 19.71 mmol) and conc. HCl (25 mL) were added at 0 °C and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was diluted with water and extracted with EtOAc (3 x 60 mL), and the combined organic phases were dried, filtered and concentrated to give 2.59 g (quantitative yield) of the title compound as a yellow solid.

MS (ESI$^+$) *m/z* 395 $[M+H]^+$.

(c) (2*R*)-2-{[5-(Benzylthio)-2-methoxy[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}pentan-1-ol

The product from step (b) (2.59 g, 6.57 mmol) was dissolved in MeOH (80 mL). KOH (737 mg, 13.14 mmol) was added and the reaction mixture was stirred for 1.5 h at 50 °C. After cooling to RT, the MeOH was removed under reduced pressure, the residue was diluted with brine and extracted with EtOAc (3 x 50 mL), and the combined organic phases were dried, filtered and concentrated to give 2.56 g (quantitative yield) of title compound as a yellow solid.

MS (ESI$^+$) *m/z* 391 $[M+H]^+$.

(d) 5-(Benzylthio)-7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from step (c) (2.56 g, 6.57 mmol) was dissolved in dioxane (50 mL). Conc. HCl (544 µL, 6.57 mmol) was added and the reaction mixture was stirred for 4 h at 50 °C. After cooling to RT, about half of the dioxane was removed under reduced pressure. The residue was diluted with brine, extracted with EtOAc (3 x 50 mL), and the combined

organic phases were dried and concentrated to give 2.2 g (89%) of the title compound as a brown solid. It was used in the subsequent step without further purification.
MS (ESI$^+$) *m/z* 377 $[M+H]^+$.

(e) 5-(Benzylsulfonyl)-7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from step (d) (1360 mg, 3.61 mmol) was dissolved in CH_3CN (85 mL) and water (56 mL). Potassium peroxymonosulfate (Oxone, 4 g, 6.51 mmol) was added and the resulting inhomogeneous mixture was stirred at RT for 24 h. The reaction mixture was concentrated to about one fifth of the original volume and extracted with EtOAc (3 x 40 mL). The combined organic phases were dried, filtered and concentrated to give 1.46 g (99%) of the title compound as a pale yellow powder.
MS (ESI$^+$) *m/z* 409 $[M+H]^+$.

(f) 5-(Benzyloxy)-7-{[(1*R*)-1-(hydroxymethyl)butyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

NaH (17 mg, 0.71 mmol) was added to a slurry of the product from step (e) (29 mg, 0.071 mmol) and benzyl alcohol (77 mg, 0.71 mmol)) in dry benzene (0.5 mL) at RT. The reaction mixture was stirred for a few minutes at RT, and then heated to 40 °C for 50 min. After cooling to RT, the reaction mixture was quenched with water (0.1 mL) and concentrated. The residue was dissolved in DMSO (1 mL) and then purified by preparative HPLC to give 13.5 mg (52.7%) of the title compound as an off-white solid.
^{1}H NMR (DMSO-d_6) δ 12.25 (s, 1H), 7.44-7.29 (m, 5H), 5.29 (d, 1H), 5.25 (d, 1H), 4.65 (t, 1H), 4.13 (br s, 1H), 3.46-3.40 (m, 1H), 1.61-1.51 (m, 1H), 1.46-1.14 (m, 3H), 0.84 (t, 3H);
MS (ESI$^+$) *m/z* 361 $[M+H]^+$.

Example 5 7-{[(1*R*)-1-(Hydroxymethyl)butyl]amino}-5-{[(1*S*)-1-phenylethyl]oxy}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from Example 4, step (e) (62 mg, 0.15 mmol) and (*S*)-1-phenylethanol (185 mg, 1.51 mmol) were dissolved in dry THF (2 mL) at RT, and n-BuLi (1.6M in hexanes, 0.85 mL, 1.36 mmol) was added. After stirring for 15 min at RT, the reaction mixture was

heated to 50 °C for 24 h, cooled to RT and concentrated. The residue obtained was dissolved in DMSO (1 mL) and then purified by preparative HPLC to give 11.4 mg (20%) of the title compound as a slightly yellowish oil.

^{1}H NMR ($CDCl_3$) δ 7.41-7.23 (m, 5H), 5.90 (q, 1H), 4.60 (br d, 1H), 4.21-12 (m, 1H), 3.48 (dd, 1H), 3.42 (dd, 1H), 2.09 (s, 3H), 1.65-1.37 (m, 4), 0.97 (t, 3H);

MS (ESI$^+$) *m/z* 375 $[M+H]^+$.

Example 6 *N*-(3-{[(7-{[(1*R*)-1-(Hydroxymethyl)butyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)-*N*-methylmethanesulfonamide

(a) Methyl 3-[methyl(methylsulfonyl)amino]benzoate

Solid NaOMe (260 mg, 4.79 mmol) was added to a solution of methyl 3-[(methylsulfonyl)amino]benzoate (Laurence, C.; Berthelot, M.; Lucon, M.; Tsuno, Y. *Spectrochim. Acta Part A* **1982**, *38*, 791-796) (500 mg, 2.18 mmol) and MeI (0.4 mL, 6.42 mmol) in a mixture of THF (15 mL) and MeOH (15 mL). After 1 h at RT, the reaction mixture was heated to 50 °C for 1.5 h. The reaction mixture was cooled to RT, diluted with brine (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated, and the residue was purified by preparative HPLC to give 436 mg (82.2%) of the title compound as a white solid.

^{1}H NMR ($CDCl_3$) δ 8.00-7.91 (m, 2H), 7.60 (d, 1H), 7.44 (t, 1H), 3.89 (s, 3H), 3.33 (s, 3H), 2.83 (s, 3H);

MS (ESI$^+$) *m/z* 244 $[M+H]^+$.

(b) *N*-[3-(Hydroxymethyl)phenyl]-*N*-methylmethanesulfonamide

Lithium borohydride (195 mg, 8.96 mmol) was added to a solution of the product from step (a) (436 mg, 1.79 mmol) in THF (25 mL). The reaction mixture was stirred for 2 h at RT, and then 20 h at 50 °C. After cooling to RT, the mixture was diluted with brine (30 mL) and extracted with EtOAc (2 x 40 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (0-5% MeOH in $CHCl_3$) to give 360 mg (93%) of the title compound as colourless oil.

^{1}H NMR ($CDCl_3$) δ 7.31-7.27 (m, 2H), 7.21-7.16 (m, 2H), 4.57 (s, 2H), 3.22 (s, 3H), 2.75 (s, 3H);
MS (ESI$^+$) *m/z* 216 $[M+H]^+$.

(c) *N*-(3-{[(7-{[(1*R*)-1-(Hydroxymethyl)butyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)-*N*-methylmethanesulfonamide

n-BuLi (0.175 mL, 0.28 mmol, 1.6M in hexanes) was added to a stirred solution of *N*-[3-(hydroxymethyl)phenyl]-*N*-methyl-methanesulfonamide (from step (b), 60 mg, 0.28 mmol) and the product from Example 4, step (e) (36.5 mg, 0.089 mmol) in dry THF (1 mL). The resulting mixture was stirred at 50 °C for 18 h. After cooling to RT, the reaction mixture was concentrated, and the residue dissolved in DMSO (0.5 mL) and then purified by preparative HPLC to give 4 mg (9.6%) of the title compound as a white solid.
MS (ESI$^+$) *m/z* 468 $[M+H]^+$.

Example 7 *N*-(3-{[(7-{[(1*R*)-1-(Hydroxymethyl)-2-methylpropyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)-methanesulfonamide

(a) (2*R*)-2-{[5-(Benzylthio)-2-chloro[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}-3-methylbutan-1-ol

A suspension of (2*R*)-2-{[2-amino-5-(benzylthio)[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}-3-methylbutan-1-ol (WO 02/76990) (4.00 g, 10.7 mmol) in conc. HCl (150 mL) and CH_3CN (110 mL) was cooled to 0 °C. Sodium nitrite (1.47 g, 21.3 mmol) was added and the solution was stirred at 0 °C for 1 h. Water (640 mL) was added and the resulting mixture was stirred for 15 min followed by filtration of the precipitate. The solid was washed with water and dried *in vacuo* over P_2O_5 at RT for 48 h resulting in 3.54 g (84%) of the title compound as a pink solid.
^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1H), 7.44-7.39 (m, 2H), 7.32-7.26 (m, 2H), 7.25-7.19 (m, 1H), 4.81-4.49 (br s, 1H), 4.39 (d, 1H), 4.34 (d, 1H), 4.14-4.05 (m, 1H), 3.57-3.45 (m, 2H), 1.98-1.87 (m. 1H), 0.92-0.80 (m, 6H);
MS (ESI$^+$) *m/z* 395 $[M+H]^+$.

(b) (2*R*)-2-{[5-(Benzylthio)-2-methoxy[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}-3-methylbutan-1-ol

Using the product of step (a) as starting material, the title compound was obtained as a beige solid (67%) by following the general method described in Example 1, step (b).

^{1}H NMR (DMSO-d_6) δ 7.61-7.54 (m, 1H), 7.45-7.36 (m, 2H), 7.35-7.19 (m, 3H), 4.66-4.58 (m, 1H), 4.41-4.30 (m, 2H), 4.19-4.02 (m, 4H), 3.58-3.43 (m, 2H), 1.97-1.86 (m. 1H), 0.92-0.80 (m, 6H);

MS (ESI$^+$) *m/z* 391 $[M+H]^+$.

(c) 5-(Benzylthio)-7-{[(1*R*)-1-(hydroxymethyl)-2-methylpropyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Using the product of step (b) as starting material, the title compound was obtained as a light orange solid (68%) by following the general method described in Example 1, step (c).

^{1}H NMR (DMSO-d_6) δ 12.36 (br s, 1H), 7.43-7.38 (m, 2H), 7.32-7.19 (m, 4H), 4.57 (app t, 1H), 4.33 (d, 1H), 4.28 (d, 1H), 4.08-3.97 (m, 1H), 3.54-3.41 (m, 2H), 1.93-1.83 (m. 1H), 0.87-0.79 (m, 6H);

MS (ESI$^+$) *m/z* 377 $[M+H]^+$.

(d) 5-(Benzylsulfonyl)-7-{[(1*R*)-1-(hydroxymethyl)-2-methylpropyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Using the product of step (c) as starting material, the title compound was obtained as a pale yellow powder (99%) by following the general method described in Example 1, step (d).

MS (ESI$^+$) *m/z* 409 $[M+H]^+$.

(e) *N*-(3-{[(7-{[(1*R*)-1-(Hydroxymethyl)-2-methylpropyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}phenyl)methanesulfonamide

Solid sodium hydride (18 mg, 0.75 mmol) was added to a stirred solution of the product of step (d) (28.5 mg, 0.069 mmol) and *N*-[3-(hydroxymethyl)phenyl]-methanesulfonamide (WO 01/90070) (35 mg, 0.17 mmol) in a mixture of toluene (0.2 mL) and 1-methyl-2-pyrrolidinone (0.2 mL) at RT. The reaction mixture was stirred for 16 h at 50 °C. After cooling to RT, the reaction mixture was quenched with water (0.1 mL) and concentrated.

The residue was dissolved in DMSO (1 mL), and purified by preparative HPLC to give 4.5 mg (14.3%) of the title compound as an off-white solid.
MS (ESI$^+$) *m/z* 454 $[M+H]^+$.

Example 8 5-(Benzyloxy)-7-{[1-(hydroxymethyl)cyclopentyl]amino}-[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

a) (1-{[2-Amino-5-(benzylthio)[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}cyclopentyl)methanol

The title compound was prepared using the general method of Example 4, step (a), but replacing 2-amino-(2*R*)-1-pentanol with cycloleucinol. The yellow solid was collected by filtration, washed with water and used for the next step without further purification.
MS (ESI$^+$) *m/z* 388 $[M+H]^+$.

b) 5-(Benzylthio)-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The product from step (a) (1.2 g, 3.1 mmol) was suspended in water (150 mL), DMSO (10 mL) was added, and the mixture was heated to 80 °C. Solid sodium nitrite (2.14 g, 31 mmol) was added in one portion and the mixture was heated at 80 °C for 3 h. After cooling to RT, acetic acid (10 mL) was added, and the white precipitate was collected by filtration. Purification of the crude product by flash column chromatography (EtOAc:CH_2Cl_2 30:70) afforded the title compound (288 mg, 24% over two steps) as a white solid.
^{1}H NMR (DMSO-d_6) δ 12.45 (s, 1H), 7.44-7.22 (m, 5H), 7.0 (br s, 1H), 4.77 (t, 1H), 4.33 (s, 2H), 3.63 (d, 2H), 1.98 (m, 2H), 1.75 (m, 2H), 1.60 (m, 2H), 1.49 (m, 2H);
MS (ESI$^+$) *m/z* 389 $[M+H]^+$.

c) 5-(Benzylsulfonyl)-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was prepared from the product of step (b), by following the procedure used in Example 1, step (d), and was obtained as an off-white solid in 86% yield.
^{1}H NMR (DMSO-d_6) δ 12.45 (s, 1H), 7.45-7.22 (m, 5H), 7.11 (br s, 1H), 4.93 (t, 1H), 4.82 (s, 2H), 3.60 (d, 2H), 1.98 (m, 2H), 1.72 (m, 2H), 1.60 (m, 2H), 1.46 (m, 2H);

MS (ESI$^+$) *m/z* 421 $[M+H]^+$.

(d) 5-(Benzyloxy)-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Solid sodium hydride (17 mg, 0.7 mmol) was added to a stirred mixture of benzyl alcohol (ca. 850 µL) and toluene (ca. 150 µL) at 60 °C. The solution was stirred at that temperature for 15 min, then the product of step (c) (42 mg, 0.1 mmol; 1 eq) was added as a solid in one portion, and the mixture was stirred at 60 °C for 1 h. After cooling to RT, the reaction was quenched by addition of saturated aqueous NH_4Cl (1 mL). The mixture was then partitioned between THF (10 mL) and water (10 mL). The organic phase was separated, dried, and concentrated. The residual oil was then triturated with EtOAc:hexane 1:1 (about 15 mL). The residue was purified by preparative HPLC to give the title compound as an off-white crystalline solid (16% yield).

^{1}H NMR (DMSO-d_6) δ 12.50 (s, 1H), 7.44-7.22 (m, 5H), 7.11 (br s, 1H), 5.13 (s, 2H), 4.90 (t, 1H), 3.71 (d, 2H), 1.98 (m, 2H), 1.72 (m, 2H), 1.60 (m, 2H), 1.45 (m, 2H);
MS (ESI$^+$) *m/z* 373 $[M+H]^+$.

The compounds of Examples 9 to 16 were prepared using the general method of Example 8, step (d), but replacing benzyl alcohol with the appropriate alcohol.

Example 9 7-{[1-(Hydroxymethyl)cyclopentyl]amino}-5-[(2-methylbenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Off-white solid (6.5 mg, 17% yield).

^{1}H NMR (DMSO-d_6) δ 12.55 (s, 1H), 7.40 (d, 1H), 7.35-7.22 (m, 3H), 7.08 (br s, 1H), 5.13 (s, 2H), 4.85 (t, 1H), 3.73 (d, 2H), 2.33 (s, 3H), 2.00 (m, 2H), 1.72 (m, 2H), 1.63 (m, 2H), 1.45 (m, 2H);
MS (ESI$^+$) *m/z* 387 $[M+H]^+$.

Example 10 7-{[1-(Hydroxymethyl)cyclopentyl]amino}-5-[(3-methylbenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Off-white solid (6.5 mg, 17% yield).

^{1}H NMR (DMSO-d_6) δ 12.52 (s, 1H), 7.37-7.20 (m, 3H), 7.05 (br s, 1H), 6.92 (d, 1H), 5.19 (s, 2H), 4.82 (t, 1H), 3.70 (d, 2H), 2.38 (s, 3H), 2.00 (m, 2H), 1.76 (m, 2H), 1.63 (m, 2H), 1.43 (m, 2H);
MS (ESI$^+$) *m/z* 387 $[M+H]^+$.

Example 11 5-[(2-Chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (5.7 mg, 14% yield).
^{1}H NMR (DMSO-d_6) δ 12.42 (s, 1H), 7.31-7.05 (m, 4H), 7.05 (br s, 1H), 5.09 (s, 2H), 4.80 (t, 1H), 3.70 (d, 2H), 1.93 (m, 2H), 1.75 (m, 2H), 1.60 (m, 2H), 1.43 (m, 2H);
MS (ESI$^+$) *m/z* 406 $[M+H]^+$.

Example 12 5-[(3-Chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (6.1 mg, 15% yield).
^{1}H NMR (DMSO-d_6) δ 12.48 (s, 1H), 7.30 (s, 1H), 7.21-7.05 (m, 3H), 7.01 (br s, 1H), 5.12 (s, 2H), 4.81 (t, 1H), 3.75 (d, 2H), 1.97 (m, 2H), 1.75 (m, 2H), 1.63 (m, 2H), 1.41 (m, 2H);
MS (ESI$^+$) *m/z* 406 $[M+H]^+$.

Example 13 5-[(4-Chlorobenzyl)oxy]-7-{[1-(hydroxymethyl)cyclopentyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (6.1 mg, 15% yield).
^{1}H NMR (DMSO-d_6) δ 12.28 (s, 1H), 7.37 (d, 2H), 7.14 (d, 2H), 6.90 (br s, 1H), 5.22 (s, 2H), 4.88 (t, 1H), 3.75 (d, 2H), 1.99 (m, 2H), 1.75 (m, 2H), 1.64 (m, 2H), 1.40 (m, 2H);
MS (ESI$^+$) *m/z* 406 $[M+H]^+$.

Example 14 7-{[1-(Hydroxymethyl)cyclopentyl]amino}-5-[(2-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
Off-white solid (4.8 mg, 12% yield).
^{1}H NMR (DMSO-d_6) δ 12.17 (s, 1H), 7.34 (d, 1H), 7.20 (m, 1H), 6.97-6.89 (3H), 5.31 (s, 2H), 4.78 (t, 1H), 3.90 (s, 3H), 3.75 (d, 2H), 1.96 (m, 2H), 1.75 (m, 2H), 1.67 (m, 2H), 1.41 (m, 2H);

MS (ESI$^+$) *m/z* 403 $[M+H]^+$.

Example 15 7-{[1-(Hydroxymethyl)cyclopentyl]amino}-5-[(3-methoxybenzyl)oxy][1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

Off-white solid (7.2 mg, 18% yield).

^{1}H NMR (DMSO-d_6) δ 12.30 (s, 1H), 7.31-7.25 (m, 2H), 7.06 (d, 1H), 6.92 (s, 1H), 6.88 (br s, 1H), 5.31 (s, 2H), 4.78 (t, 1H), 3.95 (s, 3H), 3.70 (d, 2H), 1.99 (m, 2H), 1.75 (m, 2H), 1.63 (m, 2H), 1.40 (m, 2H);

MS (ESI$^+$) *m/z* 403 $[M+H]^+$.

Example 16 4-{[(7-{[1-(Hydroxymethyl)cyclopentyl]amino}-2-oxo-2,3-dihydro[1,3]thiazolo[4,5-*d*]pyrimidin-5-yl)oxy]methyl}benzonitrile

Off-white solid (5.2 mg, 13% yield).

^{1}H NMR (DMSO-d_6) δ 12.28 (s, 1H), 7.57 (d, 2H), 7.44 (d, 2H), 6.90 (br s, 1H), 5.37 (s, 2H), 4.80 (t, 1H), 3.75 (d, 2H), 2.02 (m, 2H), 1.73 (m, 2H), 1.60 (m, 2H), 1.40 (m, 2H);

MS (ESI$^+$) *m/z* 398 $[M+H]^+$.

Example 17 (*R,S*)-7-[[1-(Hydroxymethyl)cyclopentyl]amino]-5-(1-phenylethoxy)-thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

n-BuLi (0.405 mL, 0.648 mmol, 1.6M in hexanes) was added to a stirred solution of racemic 1-phenyl-ethanol (87 mg, 0.72 mmol) in dry THF (0.2 mL) at RT. After 5 min stirring this mixture was added dropwise to the product of Example 8, step (c) (15.2 mg, 0.036 mmol) in dry THF (0.4 mL). When the addition was finished, the reaction mixture was stirred at 50 °C for 18 h. After cooling to RT, the reaction mixture was concentrated, and the residue dissolved in DMSO (1 mL) and then purified by preparative HPLC to give 3.3 mg (24%) of the title compound as a white solid.

MS (ESI$^+$) *m/z* 387 $[M+H]^+$.

Example 18 7-{[1-(Hydroxymethyl)cyclopentyl]amino}-5-{[(1*S*)-1-phenylethyl]oxy}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was prepared (7% yield) using the general method of Example 17, but replacing racemic 1-phenyl-ethanol with (1*S*)-1-phenyl-ethanol.

MS (ESI$^+$) *m/z* 387 $[M+H]^+$.

Example 19 5-{[2-(3-Chlorophenyl)-(R_S,S_S)-ethyl]sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) (2*R*)-2-{2-Chloro-5-[2-chloro-7-((1*R*)-1-hydroxymethyl-3-methyl-butylamino)-thiazolo[4,5-*d*]pyrimidin-5-yldisulfanyl]-thiazolo[4,5-*d*]pyrimidin-7-ylamino}-4-methyl-pentan-1-ol

To a slurry of (2*R*)-2-[[2-amino-5-mercapto[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino]-4-methylpentan-1-ol (WO 02/76990) (7.50 g, 25 mmol) in a mixture of conc. HCl and CH_3CN (1:1, 300 mL) at 0 °C was added dropwise a solution of sodium nitrite (5.19 g, 75 mmol) in water (25 mL). The reaction mixture was stirred for 18 h at 0-5 °C, and then poured onto ice (500 mL), and extracted with EtOAc with any remaining solid being filtered off. The combined organic phases were washed sequentially with saturated NaCl and saturated aqueous $NaHCO_3$ solution. The organic phase was dried and evaporated and the solid previously filtered off was added to this. The total solid was slurried in EtOAc which, after filtration, provided the title compound (6.3 g, 80%) as a pale yellow solid.
^{1}H NMR (400 MHz, DMSO-d_6; integrals are for the monomeric unit) δ 7.98 (d, 1H), 4.47 (t, 1 H), 3.99 (br s, 1 H), 3.19-3.14 (m, 2 H), 1.31-1.15 (m, 2 H), 1.02-0.94 (m, 1 H), 0.48 (d, 3 H), 0.30 (d, 3 H);
MS (ESI$^+$) *m/z* 635 $[M+H]^+$.

(b) (2*R*)-2-{5-[7-((1*R*)-1-Hydroxymethyl-3-methyl-butylamino)-2-methoxy-thiazolo[4,5-*d*]pyrimidin-5-yldisulfanyl]-2-methoxy-thiazolo[4,5-*d*]pyrimidin-7-ylamino}-4-methyl-pentan-1-ol

To a solution of the product from step (a) (3.0 g, 4.7 mmol) in dry MeOH (200 mL) was added KOH (0.53 g, 9.4 mmol) dissolved in dry MeOH (5 mL). The reaction was maintained at 0-5 °C for 18 h. The solvent was evaporated and the residue taken up in MeOH/EtOAc (1:1). This solution was rapidly chromatographed (silica, EtOAc) to provide the title compound (2.0 g, 68%) as a white solid.
MS (ESI$^+$) *m/z* 627 $[M+H]^+$.

(c) 5-[7-{[(1*R*)-1-(Hydroxymethyl)-3-methylbutyl]amino}-[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one-5-yldisulfanyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

To a solution of the product from step (b) above (1.5 g, 2.4 mmol) in 1,4-dioxane (20 mL) was added a mixture of conc. HCl and water (40 mL, 1:1). The solution was then stirred at 45 °C for 18 h. The solvent was evaporated and the residue taken up in EtOAc (undissolved residue was filtered off and was found to be pure by LCMS). The solution was subjected to flash chromatography (silica, MeOH:EtOAc 5:95). The two samples were pooled together to give a white solid (600 mg, 42%, 75% pure by HPLC). This material was used without further purification in the ensuing reactions.

^{1}H NMR (DMSO-d_6; integrals are for the monomeric unit) δ 12.45 (s, 2H), 7.33 (d, 2H), 4.62 (t, 2H), 4.17 (br s, 2H), 1.48-1.31 (m, 4H), 1.25-1.14 (m, 2H), 0.72 (d, 6H), 0.56 (d, 6H);

MS (ESI$^+$) *m/z* 599 $[M+H]^+$.

(d) 1-(2-Bromoethyl)-3-chlorobenzene

To a solution of 2-(3-chlorophenyl)ethanol (1.06 g, 6.0 mmol) in CH_2Cl_2 (50 mL) at RT under nitrogen was added CBr_4 (1.98 g, 5.8 mmol) and PPh_3 (1.57 g, 5.8 mmol). After stirring at RT for 18 h the reaction mixture was concentrated and the residue diluted with Et_2O (30 mL) resulting in precipitation of triphenylphosphine oxide. The ethereal solution was decanted, evaporated and purified *via* flash chromatography (silica, hexane) to provide 2-(3-chloro)phenylethyl bromide as a clear oil (57%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39-7.22 (m, 3 H), 7.18-7.09 (m, 1 H), 3.63-3.51 (m, 2 H), 3.25-3.17 (m, 2 H);

^{13}C NMR (100.6 MHz, DMSO-d_6) δ 141.2, 134.6, 130.7, 129.3, 127.6, 127.3.

(e) 5-{[2-(3-Chlorophenyl)ethyl]thio}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

To a stirred solution of the product from step (c) above (30.0 mg, 0.05 mmol) in DMSO (0.5 mL) at RT was added $NaBH_4$ (5.6 mg, 0.125 mmol). Once effervescence had ceased, the product from step (d) above was added (20 mg, 0.09 mmol). The reaction was complete

after 18 h at RT. Purification was achieved using preparative HPLC to give a white solid (90%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38-7.02 (m, 5 H), 6.08 (br s, 1 H), 4.29 (br s, 1 H), 3.60 (dd, 1 H), 3.49 (dd, 1 H), 3.26-3.18 (m, 2 H), 2.92 (t, 2 H), 1.63-1.55 (m, 1 H), 1.46-1.31 (m, 2 H), 0.82 (d, 3 H), 0.81 (d, 3 H);

MS (ESI$^+$) *m/z* 439 [M+H]$^+$.

(f) 5-{[2-(3-Chlorophenyl)ethyl]-(R_S,S_S)-sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

To a stirred solution of the product from step (e) above (15 mg, 0.025 mmol) in MeOH (2 mL) at RT was added potassium peroxymonosulfate (Oxone, 20.5 mg, 0.033 mmol). After 1.5 h the reaction was quenched by addition of water and saturated aqueous $Na_2S_2O_3$. The aqueous phase was extracted with EtOAc, dried and evaporated. Purification was achieved using preparative HPLC to give the title compound as a white solid (mixture of two unresolved diastereoisomers, 1:1; 27%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19-7.01 (m, 8 H), 6.99-6.98 (m, 2 H), 4.52 (t, 2 H), 4.07 (br s, 2 H), 2.87-2.80 (m, 2 H), 2.76-2.63 (m, 2 H), 1.27-1.20 (m, 2 H), 1.19-1.04 (m, 4 H), 0.70-0.66 (12 H, m);

MS (ESI$^+$) *m/z* 455 [M+H]$^+$.

Example 20 5-{[2-(2-Bromophenyl)ethyl]-(R_S,S_S)-sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) 5-{[2-(2-Bromophenyl)ethyl]thio}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

By following the procedure in Example 19, step (e), the title compound was obtained as a white solid in 58% yield from the reaction of the product of Example 19, step (c) with 1-(2-bromoethyl)-2-bromobenzene which, in turn, was prepared from 2-(2-bromophenyl)ethanol according to the procedure described in Example 19, step (d).

^{1}H-NMR ($CDCl_3$) δ 7.55 (unresolved dd, 1H), 7.29 (dd, 1H), 7.25 (unresolved dt, 1H), 7.10 (dt, 1H), 5.1 (br s, 1H), 3.82 (dd, 1H), 3.67 (dd, 1H), 3.89 (app dt, 2H), 3.16 (t, 2H), 1.85-1.63 (m, 1H), 1.58-1.42 (m, 2H), 0.95 (d, 3H), 0.93 (d, 3H);

MS (ESI$^+$) *m/z* 483, 485 $[M+H]^+$.

(b) 5-{[2-(2-Bromophenyl)ethyl]-(R_S,S_S)-sulfinyl}-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained as a clear film in 62% yield (1:1 mixture of two unresolved diastereoisomers) from the product of step (a), by following the procedure described in Example 19, step (f).

^{1}H-NMR (CD_3OD) δ 7.50 (app dd, 1H), 7.27-7.21 (m, 2H), 7.12-7.06 (m, 1H), 4.97 (protons in the water peak, 3H) 4.45 (br s, 1H), 3.57-3.48 (m, 2H), 3.48-3.42 (m, 2H from one diastereomer), 3.37-3.30 (m, 2H from one diastereomer), 3.23-3.19 (m, 2H from one diastereomer), 3.07-3.01 (m, 2H from one diastereomer), 1.68-1.63 (m, 1H), 1.55-1.37 (m, 2H), 0.92 (d, 3H from one diastereomer), 0.90 (t, 6H from one diastereomer), 0.87 (d, 3H from one diastereomer);

MS (ESI$^+$) *m/z* 499, 501 $[M+H]^+$.

Example 21 5-[(2,3-Difluorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained as a white solid in 41% yield (1:1 mixture of two unresolved diastereoisomers) starting from the product of Example 1, step (c) by following the general procedure described in Example 19, step (f).

^{1}H NMR (DMSO-d_6) δ 12.86 (b s, 1H), 7.68 (b s, 1H) 7.45-7.32 (m, 1H), 7.20-7.10 (m, 1H), 7.05-6.90 (m 1H), 4.77 (b s, 1H), 4.62-4.48 (app t, 1H), 4.38-4.20 (m, 2H), 3.90 (2H, partially under the water peak), 1.58 (b s, 1H), 1.50-1.30 (m, 2H), 0.88 (d, 3H), 0.84 (d, 3H);

MS (ESI$^+$) *m/z* 443 $[M+H]^+$.

Example 22 5-[Benzyl-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) (2*R*)-2-{[5-(Benzylthio)-2-methoxy[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}-4-methylpentan-1-ol

To a suspension of (2*R*)-2-{[5-(benzylthio)-2-bromo[1,3]thiazolo[4,5-*d*]pyrimidin-7-yl]amino}-4-methylpentan-1-ol (1.90 g, 4.19 mmol) (WO 02/76990) in anhydrous MeOH (45 mL) was added potassium hydroxide (0.52 g, 9.22 mmol). The mixture was stirred at RT for 35 minutes followed by the addition of conc. HCl to pH 5. The solvent was evaporated and the crude solid was partitioned between water and methylene chloride. The organic phase was washed twice with water, brine, dried ($MgSO_4$), filtered, and the solvent was evaporated. The product was dried *in vacuo* at 35 °C for 2 h to give 1.72 g (quantitative yield) of the title compound as an orange solid.

^{1}H NMR ($CDCl_3$) δ 7.43 (d, 2H), 7.32-7.20 (m, 3H), 4.54 (d, 1H), 4.45-4.43 (m, 2H), 4.40-4.30 (m, 1H), 4.26 (s, 3H), 3.78-3.71 (m, 1H), 3.62-3.55 (m, 1H), 2.28 (t, 1H), 1.72-1.61 (m, 1H), 1.53-1.38 (m, 2H), 0.96-0.89 (m, 6H);

MS (ESI$^+$) *m/z* 405 $[M+H]^+$.

(b) 5-(Benzylthio)-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

To a solution of the product from step (a) (1.72 g, 4.25 mmol) in 1,4-dioxane (50 mL) and water (1 mL) was added conc. HCl (0.91 mL). The mixture was heated at 45 °C for 15 h followed by evaporation of the solvent. A mixture of EtOAc/methylene chloride (5 mL, 30:70) was added and the solution was subjected to a stream of nitrogen gas for 2.5 h. The resulting solid was filtered off and washed with methylene chloride followed by EtOAc. The mother liquor was concentrated and flash chromatographed on silica (eluent EtOAc :methylene chloride 30:70). The two products were pooled resulting in 1.11 g (67% yield) of the title compound as a white solid.

^{1}H NMR (DMSO-d_6) δ 12.35 (br s, 1H), 7.43-7.39 (m, 2H), 7.31-7.19 (m, 4H), 4.36-4.23 (m, 3H), 3.45-3.28 (m, 1H) overlapping with H_2O-signal, 1.63-1.51 (m, 1H), 1.46-1.31 (m, 2H), 0.88-0.78 (m, 6H);

MS (ESI$^+$) *m/z* 391 $[M+H]^+$.

(c) 5-[Benzyl-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained as a white solid in a 17% yield (1:1 mixture of two unresolved diastereoisomers) by following the method described in Example 19, step (f).

^{1}H NMR (DMSO-d_6) δ 12.82 (br s, 1H), 7.49 (br s, 1H), 7.34-7.26 (m, 3H), 7.17-7.10 (m, 2H), 4.76 (app t, 1H), 4.37 (dd, 2H from one diastereomer) overlapping with 4.30 (br s, 1H), 4.18 (dd, 2H from one diastereomer), 3.48-3.22 (m, 2H) overlapping with H_2O-signal, 1.59 (br s, 1H), 1.49-1.32 (m, 2H), 0.93-0.82 (m, 6H);
MS (ESI$^+$) *m/z* 407 $[M+H]^+$.

The compounds of Examples 23 to 25 were prepared using the general method of Example 19, step (f). The precursor sulfides were prepared according to the method of Example 19, step (e), but replacing 1-(2-bromoethyl)-3-chlorobenzene with the appropriate benzylic halide, all of which are commercially available.

Example 23 5-[(2-Chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) 5-[(2-Chlorobenzyl)thio]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
The title compound was obtained as a white solid in 52% yield from the product of Example 19, step (c), and 1-chloro-2-(chloromethyl)benzene.
^{1}H NMR (CD_3OD) δ 7.62-7.56 (m, 1H), 7.36-7.30 (m, 1H), 7.40-7.35 (m, 1H), 7.25-7.19 (m, 2H), 4.47 (dd, 2H) overlapping with 4.42 (br s, 1H), 3.56-3.47 (m, 2H), 1.71-1.59 (m, 1H), 1.56-1.46 (m, 1H), 1.45-1.36 (m, 1H), 0.92 (d, 3H), 0.89 (d, 3H);
MS (ESI$^+$) *m/z* 425 $[M+H]^+$.

(b) 5-[(2-Chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one
The title compound was obtained as an off-white solid in a 30% yield (1:1 mixture of two unresolved diastereoisomers).
^{1}H NMR (CD_3OD) δ 7.44 (d, 1H), 7.36-7.30 (m, 1H), 7.30-7.23 (m, 2H), 4.70 (dd, 2H from one diastereomer), 4.46 (br s, 1H), 4.37 (app t, 2H from one diastereomer), 3.58-3.47 (m, 2H), 1.72-1.60 (m, 1H), 1.55-1.38 (m, 2H), 0.97-0.88 (m, 6H);
MS (ESI$^+$) *m/z* 441 $[M+H]^+$.

Example 24 5-[(4-Chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

(a) 5-[(4-Chlorobenzyl)thio]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained as a white solid in 58% yield from the product of Example 19, step (c) and 1-chloro-4-(chloromethyl)benzene.

^{1}H NMR (CD_3OD) δ 7.41 (app d, 2H), 7.28 (app d, 2H), 4.39 (br s, 1H) overlapping with 4.34 (dd, 2H), 3.56-3.46 (m, 2H), 1.70-1.59 (m, 1H), 1.54-1.45 (m, 1H), 1.45-1.36 (m, 1H), 0.92 (d, 3H), 0.87 (d, 3H);

MS (ESI$^+$) *m/z* 425 $[M+H]^+$.

(b) 5-[(4-Chlorobenzyl)-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-3-methylbutyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained as a white solid in 25% yield (1:1 mixture of two unresolved diastereoisomers).

^{1}H NMR (CD_3OD) δ 7.28 (app t, 2H), 7.12 (app d, 2H), 4.45 (br s, 1H) overlapping with 4.42 (dd, 2H from one diastereomer), 4.27 (dd, 2H from one diastereomer), 3.58-3.47 (m, 2H), 1.72-1.57 (m, 1H), 1.55-1.35 (m, 2H), 0.98-0.86 (m, 6H);

MS (ESI$^+$) *m/z* 441 $[M+H]^+$.

Example 25 5-[Benzyl-(R_S,S_S)-sulfinyl]-7-{[(1*R*)-1-(hydroxymethyl)-2-methylpropyl]amino}[1,3]thiazolo[4,5-*d*]pyrimidin-2(3*H*)-one

The title compound was obtained from the product of Example 7, step (c) as a white solid in a 17% yield (1:1 mixture of two unresolved diastereoisomers) by following the procedure of Example 19, step (f).

^{1}H NMR (DMSO-d_6) δ 12.73 (br s, 1H), 7.63 (br s, 1H), 7.32-7.26 (m, 3H), 7.18-7.12 (m, 2H), 4.67-4.62 (m, 1H), 4.37 (dd, 2H from one diastereomer), 4.20 (d, 2H from one diastereomer), 4.12-4.02 (m, 1H), 3.60-3.45 (m, 2H), 1.95-1.85 (m, 1H), 0.93-0.83 (m, 6H);

MS (ESI+) *m/z* 393 $[M+H]^+$.

Pharmacological Screens

Materials

Recombinant human fractalkine (hCX_3CL1) was purchased from PeproTech Inc., UK. Recombinant [^{125}I]-fractalkine (human), with a specific activity of 2200 Ci/mmol, was purchased from NEN® Life Science Products, Inc., UK. Fluo4-AM was purchased from Molecular Probes, US. All other chemicals were of analytical grade.

Expression of human fractalkine receptor (hCX_3CR1)

The complete human CX3CR1 cDNA (GenBank accession number U20350) was extracted from human brain mRNA (Superscript, Life Technologies) and ligated into pCR-Blunt II TOPO vector (InVitrogen). The insert corresponding hCX3CR1 was isolated and further subcloned into pcDNA3.1zeo. Plasmid DNA was prepared using Plasmid Midi Kit (Qiagen). Using Superfect Transfection Reagent (Qiagen) according to the manufacture´s protocol the expression plasmid for hCX_3CR1 was then introduced into human embryonic kidney suspension (HEKS) 293 cell line containing a vector for stable expression of a chimeric G-protein $G\alpha_{qi5}$. A stable clone was generated utilizing zeocin (500 µg/ml) and hygromycin (100 µg/ml) selection. For further applications the cells were maintained in Dulbecco´s modified Eagle´s medium/Ham´s nutrient mix F12 (DMEM/F12) containing pyridoxine and supplemented with 10% (v/v) fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin, 250 µg/ml zeocin and 100 µg/ml hygromycin.

Ligand Binding Assay

For the competition binding assay cells were harvested in buffer containing 10 mM Tris-HCl, pH 7.4, 5 mM ethylenediaminetetra-aceticacid (EDTA) and 0.1 mg/ml bacitracin (a protease inhibitor) and centrifuged at 300xg for 10 min. Cell pellets were then resuspended in harvesting buffer, pooled and homogenised using Dounce homogeniser. Cell membranes were centrifuged at 48000xg for 10 min and then resuspended in harvesting buffer using Ultra-Turrax T8 (IKA Labortechnik, Germany). Protein concentration was determined in microtiter plates as described by Harrington (1990, Anal. Biochem. 186, 285 – 287). Membrane aliquotes were stored at –70 °C. Receptor expression was confirmed with [^{125}I]-

fractalkine binding using whole cells. Competition binding assays were performed in 2ml 96-deep-well plates (Beckman, Germany) in a total volume of 1000 µl/well. Each well contained 10 pM [^{125}I]-fractalkine and membrane equivalent to receptor concentration of 1 pM in assay buffer [50 mM Hepes-KOH, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 0,1 % (w/v) gelatin]. Test compounds were pre-dissolved in DMSO and added to reach a final concentration of 1 % (v/v) DMSO. The assay was initiated with the addition of membranes and incubated at 25°C for 24 h. Assay plates were filtrated with a Tomtec cell harvester (Tomtec, US) using ice-cold wash buffer (10mM Hepes-KOH pH 7.4, 500mM NaCl) and harvested onto printed filtermat B, GF/B (PerkinElmer LifeScience,US) presoaked in 0.3% polyetyhlenimine. MeltiLex solid scintillator (PerkinElmer LifeSciences,US) were melted onto filters and radioactivity was measured in a Wallac1205 Betaplate counter (PerkinElmer LifeScience, US).

Solubility Assay

Method Description

100 µM Solutions in duplicate, prepared by dilution from a 10 mM DMSO stock solution of the test compound, were incubated in 0.1M phosphate buffer, pH 7.4, in a 96-well plate (PP plate, 350 µl U-shaped wells, COSTAR) on a plate bed shaker (IKA®-Schüttler MTS-4, IKA Labortechnik) at 300 rpm and room temperature (20-22 °C) for 24 hours.

The solutions were transferred to a MultiScreen™-R4 96-well filtration plate (LCR membrane, 0.4 µm hydrophilic PTFE, non-sterile glass-filled PP plate, 350 µl wells, Millipore) and filtered under vacuum to a 96-well collection plate (PP plate, 350 µl U-shaped wells, COSTAR), called the analyte plate, using Millipore Vacuum Manifold equipment. The analyte plate was covered by heat-sealing with an aluminium foil coated with a PP seal layer (AB-0813, pierceable sealing foil strong, ABgene).

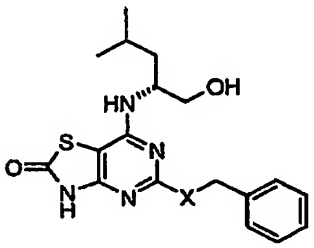
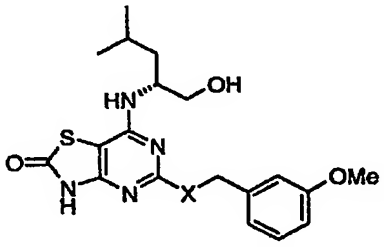
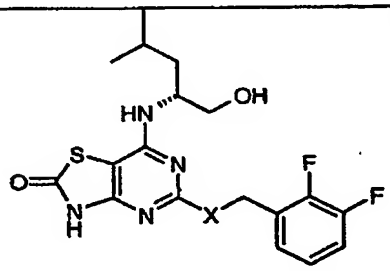
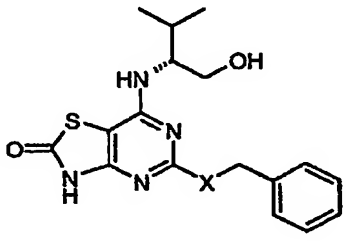
LC-UV-MS analysis was performed using a generic LC method.

Single point quantification was performed against two 100 µM standards of the test compound dissolved in DMSO at the wavelength showing maximum UV absorbance as

extracted from the DAD-trace (210 - 400 nm). The upper limit of the screen method is 100 μM with a LOQ of 0.1 μM.

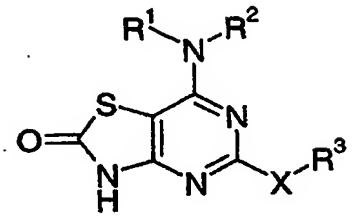
Results

When tested in the ligand binding assay, the compounds of Examples 1 to 25 gave K_i values of less than 10 μM, indicating that they are expected to show useful therapeutic activity. Representative ligand binding and solubility data are shown in the following Table in which four Examples from the present application are compared with the corresponding sulphide derivatives (X = S) from within the generic scope of WO 01/25242:

Compound		Solubility (μM)	CX_3CR1 Receptor K_i (nM)
	X = O Example 1	29	44.6
	X = S	1.3	50.0
	X = O Example 2	78.5	53.1
	X = S	3.5	45.0
	X = S(O) Example 21	> 100	38.0
	X = S	2.1	10.0
	X = S(O) Example 25	> 100	286.7
	X = S	8.5	179.1

Claims

1. A compound of formula (I)



(I)

wherein:

R^1 and R^2 independently represent H, C1 to 8 alkyl, C2 to 8 alkenyl, C2 to 8 alkynyl or C3 to 7 saturated or partially unsaturated cycloalkyl; the latter four groups being optionally further substituted by one or more groups selected independently from OH, C1 to 6 alkoxy, CH_2OR^4, NR^5R^6, CO_2R^7 and $CONR^8R^9$;

R^3 represents C1 to 6 alkyl, C2 to 6 alkenyl, C2 to 6 alkynyl or C3 to 7 saturated or partially unsaturated cycloalkyl; said alkyl, alkenyl or alkynyl chain optionally including a O, NR^{10} or S atom in the chain; said alkyl, alkenyl, alkynyl or cycloalkyl group being optionally substituted by phenyl or a 5 or 6 membered heteroaromatic ring containing 1 to 3 heteroatoms selected independently from O, S and N; said phenyl or heteroaromatic ring being optionally further substituted by one or more groups selected independently from halogen, C1 to 4 alkyl, OH, C1 to 4 alkoxy, CN, $CO2R^{11}$, $NR^{12}R^{13}$, $CONR^{14}R^{15}$, SO_2R^{16}, $NR^{17}SO_2R^{18}$ and $SO_2NR^{19}R^{20}$;

X represents O or S(O);

R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{20} independently represent H or C1 to 6 alkyl;

and pharmaceutically acceptable salts thereof.

2. A compound according to Claim 1 wherein R^1 represents H or CH_3.

3. A compound according to Claim 1 or Claim 2 wherein R^2 represents C1 to 8 alkyl substituted by OH or C3 to 7 cycloalkyl substituted by OH or CH_2OR^4.

4. A compound according to any one of Claims 1 to 3 wherein R3 represents C1 to 2 alkyl substituted by phenyl; said phenyl being optionally substituted by halogen, C1 to 6 alkoxy or CN.

5. A compound of formula (I), according to any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, for use as a medicament.

6. A pharmaceutical formulation comprising a compound of formula (I), as defined in any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, optionally in admixture with a pharmaceutically acceptable diluent or carrier.

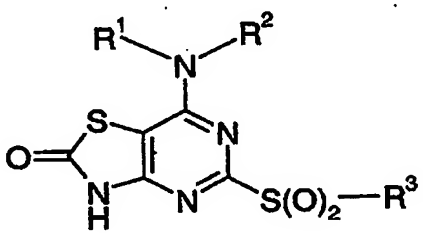
7. A method of treating, or reducing the risk of, a human disease or condition in which antagonism of the CX_3CR1 receptor is beneficial which comprises administering to a person suffering from or susceptible to such a disease or condition, a therapeutically effective amount of a compound of formula (I), as defined in any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof.

8. The use of a compound of formula (I) as defined in any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment or prophylaxis of human diseases or conditions in which antagonism of the CX_3CR1 receptor is beneficial.

9. The use of a compound of formula (I) as defined in any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment or prophylaxis of neurodegenerative disorders, demyelinating disease, atherosclerosis or pain.

10. A process for the preparation of a compound of formula (I), as defined in any one of Claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein the process comprises:

(a) when X in formula (I) represents O, reaction of a compound of formula (II)



(II)

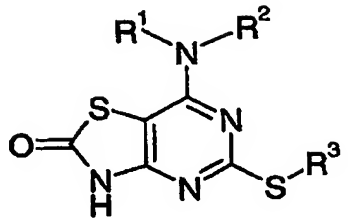
wherein R^1, R^2 and R^3 are as defined in Claim 1;

with a compound of formula (III)

(III)

wherein R^3 is as defined in Claim 1 and is independent of the R^3 group in formula (II); or

(b) when X in formula (I) represents S(O), oxidation of a compound of formula (IV)



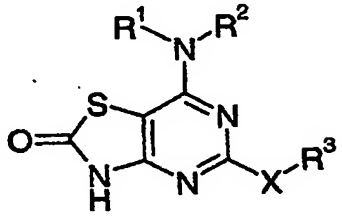
(IV)

wherein R^1, R^2 and R^3 are as defined in Claim 1; with one equivalent of an oxidising agent;

and where necessary converting the resultant compound of formula (I), or another salt thereof, into a pharmaceutically acceptable salt thereof; or converting the resultant compound of formula (I) into a further compound of formula (I); and where desired converting the resultant compound of formula (I) into an optical isomer thereof.

Abstract

There are disclosed novel compounds of formula (I)



(I)

wherein R^1, R^2, R^3 and X are as defined in the specification, and pharmaceutically acceptable salts thereof, together with processes for their preparation, pharmaceutical compositions comprising them and their use in therapy. The compounds of formula (I) are CX_3CR1 receptor antagonists and are thereby particularly useful in the treatment or prophylaxis of neurodegenerative disorders, demyelinating disease, atherosclerosis and pain.